A comparison of dry matter yield stability assessment methods for small numbers of genotypes of bermudagrass

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Abstract   No information is available on the efficacy of various nonparametric stability parameters when compared with GGE biplot methodology in assessing the stability of dry matter yield in bermudagrass (*Cynodon dactylon* L. Pers.) when a small number of genotypes is assayed. This study was conducted to compare the results of four nonparametric stability parameters developed by Huehn and Nassar ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$), Kang's rank-sum method and the GGE biplot method for five genotypes over 11 location–year environments at Oklahoma State University experiment stations. Results from analysis of variance procedures indicated highly significant levels of genotype-by-environment interaction ($P < 0.01$), which further indicated the need for stability analysis measures to be conducted. Results of the stability analysis indicated agreement among $S_i^{(1)}$, $S_i^{(2)}$, Kang's rank-sum method, and the biplot method for the stability rankings of the genotypes and between these methods and the overall yield rankings of the genotypes. The $S_i^{(3)}$ and $S_i^{(6)}$ statistics were not in agreement with each other or any of the previously mentioned methods concerning the stability rankings of the genotypes. From examination of the formulae for the nonparametric statistics it was concluded that, when a small number of genotypes is assayed, the $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$ statistics have the potential to be extremely sensitive and to produce misleading results. It was further concluded that for assessment of small numbers of genotypes the GGE biplot stability analysis method, augmented with Kang's rank-sum method, would produce the most reliable estimates of genotype stability.

Keywords   Bermudagrass · Biplot · GE interaction · Nonparametric stability · Stability

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Abbreviations

GE	Genotype-by-environment
GGE	Genotype genotype-by-environment
PC	Principal component
MET	Multi-environmental trial
ERS	Eastern Research Station
SCRS	South Central Research Station

Introduction

Bermudagrass, (*Cynodon dactylon* L. Pers.), is widely used in Oklahoma and throughout the southern USA as

a forage for livestock. The characterization of bermudagrass (cultivars and genotypes) for important quantitative traits (e.g. dry matter (dm) yield) is complicated by the frequent occurrence of genotype-by-environment (GE) interactions (Avis et al. 1980; Chakroun et al. 1990). GE interactions are expressed as changes in the relative ranking of the genotypes or changes in the magnitudes of differences between genotypes from one environment to another (Nguyen et al. 1980). Significant GE interactions for quantitative traits reduces the usefulness of the genotype means over all locations or environments for selecting and advancing superior genotypes to subsequent stages of selection (Pham and Kang 1988). Furthermore, GE interaction reduces the correlation between phenotypic and genotypic values and has been shown to reduce progress from selection (Pham and Kang 1988).

Analysis of variance (ANOVA) methods were developed in the mid-1900s to detect GE interactions and provide a measure of their magnitudes (Sprague and Federer 1951; Comstock and Robinson 1952; Hanson et al. 1956; Comstock and Moll 1963). Nguyen et al. (1980) pointed out that these analyses provide no measurement of the response by individual genotypes to environments and, therefore, provide no measure of the performance stability of genotypes across environments. Additional efforts focused on developing statistical measures of performance stability of genotypes across environments using regression analyses (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Shukla 1972; Lin et al. 1986).

Huehn (1996) categorized the study of GE interactions and determination of adaptations of genotypes into two major approaches. The first and most commonly used approach is a parametric method that relies on distributional assumptions about genotypic, environmental and GE effects. The second approach is a nonparametric method that relates environments and phenotypes relative to biotic and abiotic environmental factors without considering specific modeling assumptions. However, most breeding programs incorporate some elements of both approaches for practical applications (Becker and Leon 1988). Parametric stability methods have good properties under certain statistical assumptions such as normal distribution of errors and interaction effects, however, they may not perform well if these assumptions are violated (Huehn 1990). Thus,

parametric methods for significance of variances and variance-related measures could be very sensitive to the underlying assumptions. Therefore, it is worth exploring alternative approaches such as nonparametric approaches that are not so sensitive to common assumptions (Nassar and Huehn 1987). Several nonparametric methods for genotype stability estimates have been proposed by Huehn (1979), Nassar and Huehn (1987) and Kang (1988).

According to Huehn (1990), the nonparametric methods have the following advantages over the parametric methods: reduction or avoidance of the bias caused by outliers; no assumptions are needed about the distribution of the phenotypic values; stability parameters based on ranks are easy to use and interpret and additions or deletions of one or a few genotypes do not cause much variation of estimates. Some examples of use of nonparametric methods for genotype stability estimates in recent years are Adugna and Labuschagne (2003) and Sabaghnia et al. (2006).

More recently, data from multi-environment trials (METs) have been evaluated using biplot graphic displays based on principal component (PC) analysis to identify GE interactions as well as performance stability of cultivars and genotypes (Gabriel 1971; Kempton 1984; Gauch and Zobel 1997; Yan et al. 2000; Yan and Tinker 2005). GGE biplots are an effective tool in visualizing GE data for a trait (e.g., dm yield) and genotype-by-trait data in individual environments (Yan and Tinker 2005). Yan and Tinker (2005) noted that a single biplot simultaneously displays the genotype main effect (G) and the GE interaction of a two-way table allowing genotypes to be evaluated for their performance in individual environments or across environments, mean performance and stability, and general or specific adaptations. Yan (1999) and Yan and Kang (2003) further noted that stability analysis performed on a few environments is not informative, but further noted that stability analysis for a large number of year–location environments is redundant in that for large numbers of environments, the mean value for the trait under consideration would contain all pertinent information.

Bermudagrass is grown throughout Oklahoma under a range of climatic and edaphic conditions. Oklahoma longitudinal boundaries are 94°21′–103° W and latitudinal boundaries are 33°35′–37° N. Elevation varies from 152 m above sea level

in the extreme southeast portion of the state to 1,368 m above sea level in the western Panhandle. Mean annual precipitation varies from about 1,270 mm in the extreme southeast portion of the state to about 406 mm in the western Panhandle. Because of this environmental diversity, METs are required to characterize bermudagrass genotypes for geographic adaptation and other performance traits when grown in the state.

The length of time required to develop and test a bermudagrass cultivar is approximately 10 years (for both seed and vegetatively propagated varieties). Hence, cultivar and variety testing programs initiated each growing season may have considerable overlap with respect to cultivar and experimental entries. Data collected has indicated that, due to this overlap of cultivar and experimental entries, potentially useful information from yield trials may be extracted for a subset of a few genotypes balanced over a number of year–location environments. Valid assessment of yield stability among these genotypes could prove useful in continuous breeding efforts and in selection of cultivars by producers.

The objectives of this study were to investigate the presence of GE interaction and stability of performance for bermudagrass dm yield among five vegetatively propagated genotypes at 11 year-location environments and to assess the effectiveness of six genotype stability estimation methods when comparing a small sample of genotypes.

Materials and methods

Data collection

Data for this study were from trials employing five bermudagrass genotypes during the period 1991–1998. The respective genotypes in this study were established cultivars Hardie, Midland and Tifton 44 and experimental lines ST 10-09 and ST 15-11. The trials were conducted at two Oklahoma Agricultural Research Stations: Eastern Research Station (ERS) at Haskell, OK (35.82° N Lat., 94.50° W Long.) and the South Central Research Station (SCRS) at Chickasha, OK (35.03° N Lat., 97.95° W Long.). There were a total of 11 year–location environments for this study. The soil type at ERS was a Taloka silt loam (Fine,

mixed, active, thermic Mollic Albaqualfs) and the soil type at SCRS was a McLain silt loam (Fine, mixed, superactive, thermic Pachic Argiustolls). The experimental design used in this trial was a randomized complete block design with four replications. Plot size was 3.6×6 m. The bermudagrass tests annually received 336 kg N ha^{-1} in three applications of 112 kg ha^{-1} each; the first applied at early growth and the second and third after the first and second harvests, respectively. P and K were applied as indicated by soil test results. Yield (dm) was measured three to four times per growing season by harvesting a swath of 1 m wide by 4.56–6.1 m in length from the center of the individual plots. Samples were taken from each plot and dried for 1 week at approximately 50°C to determine moisture concentration and convert yields to a dm basis. The data used in this study were seasonal total dm yields obtained by summing dm yields over harvests within a growing season.

Statistical procedures

Yield data were initially subjected to an ANOVA employing generalized least squares in the PROC ANOVA procedure (SAS Institute 2005) in order to determine the magnitude and types of GE interactions present. The following statistical effects model was used:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \beta\tau_{jk} + e_{m(ijk)}$$

where $\mu =$ overall mean of dm yield, $\alpha_{i(k)} =$ fixed effect of replication i nested within environment k, $\beta_j =$ fixed effect of genotype j, $\tau_k =$ fixed effect of environment k, $\beta\tau_{jk} =$ fixed interaction effect of genotype j and environment k, and $e_{m(ijk)} =$ experimental error, mean 0, variance σ^2.

Five nonparametric stability parameters were used to assay the data. Four of these, proposed by Huehn (1979) and Nassar and Huehn (1987), were used and are detailed as:

$$S_i^{(1)} = 2 \sum_{j}^{n-1} \sum_{j'=j+1}^{n} |r_{ij} - r_{ij'}|/[n(n-1)]$$

$$S_i^{(2)} = \sum_{j=1}^{n} (r_{ij} - \bar{r}_{i.})^2/(n-1)$$

$$S_i^{(3)} = \frac{\sum_{j=1}^{n} \left(r_{ij} - \bar{r}_{i.} \right)^2}{\bar{r}_{i.}}$$

$$S_i^{(6)} = \frac{\sum_{j=1}^{n} \left| r_{ij} - \bar{r}_{i.} \right|}{\bar{r}_{i.}}$$

where r_{ij} = rank of the ith genotype in the jth environment and $\bar{r}_{i.}$ = mean rank across all environments for the ith genotype.

The lowest value for each of these statistics indicates maximum stability for the genotypes being compared.

The nonparametric stability statistics $S_i^{(1)}$ and $S_i^{(2)}$ are based on ranks of the genotypes across environments and they give equal weight to each environment. $S_i^{(1)}$ is based on all possible pair-wise rank differences across environments for each genotype, whereas $S_i^{(2)}$ is based on variances of ranks for each genotype across environments (Nassar and Huehn 1987). By these methods a cultivar is considered stable if its rank is similar over environments (Becker and Leon 1988). The nonparametric statistics $S_i^{(3)}$ and $S_i^{(6)}$ of Huehn (1979) combine yield and stability based on yield ranks of genotypes in each environment.

The rank-sum method of Kang (1988) was also used to assay the genotypes. It is a nonparametric stability estimation method in which both yield and Shukla's stability variance (1972) are used as selection criteria. In this method, the genotype with the highest yield is given a rank of 1; a genotype with the lowest stability variance is also given a rank of 1. All genotypes are ranked in this manner and the ranks by yield and by stability variance are added (rank-sum) for each genotype. The genotype with the lowest rank-sum is considered to be the most desirable one.

GGE biplots were constructed via a conventional method described by Yan and Kang (2003) that ascribe to the following statistical model:

$$Y_{ij} - \mu - \beta_j = g_{i1}e_{1j} + g_{i2}e_{2j} + \acute{\varepsilon}_{ij}$$

where Y_{ij} = expected yield of genotype i in environment j, μ = grand mean of dm yield, β_j = main effect of environment j, $g_{i1}e_{1j}$ = primary scores for genotype i and environment j, respectively, $g_{i2}e_{2j}$ = secondary scores for genotype i and

environment j, respectively and $\acute{\varepsilon}_{ij}$ = residue variation not explained by primary and secondary effects.

Singular value decomposition is required so that genotypic (g_{i1} and g_{i2}) and environmental (e_{1j} and e_{2j}) can be plotted against each other in the biplot. GGE biplots were produced via Excel and PowerPoint software (Microsoft 2000a, 2000b).

Interpretation of a GE biplot for mega-environment investigation is best accomplished by the formation of a polygon (convex hull) connecting the coordinates of genotypes located farthest from the biplot origin (Yan and Kang 2003) (Fig. 1). Vertex genotypes are considered to be the best-adapted genotypes for the location–year environments whose coordinates reside in a particular sector (Yan and Kang 2003) (Fig. 1). A perpendicular line originating from the biplot origin and passing through each side of the convex hull provides division of the biplot into sectors that contain genotype coordinates on the interior of the convex hull and environmental coordinates on either the interior or exterior of the convex hull (Yan and Kang 2003) (Fig. 1). Crossover GE interactions attributable to location–year interactions are manifested within the biplot as coordinates of contrasting genotypes being located within different sectors of the biplot In order to assay the stability of genotypes, the average environment coordinate (AEC) is found by taking
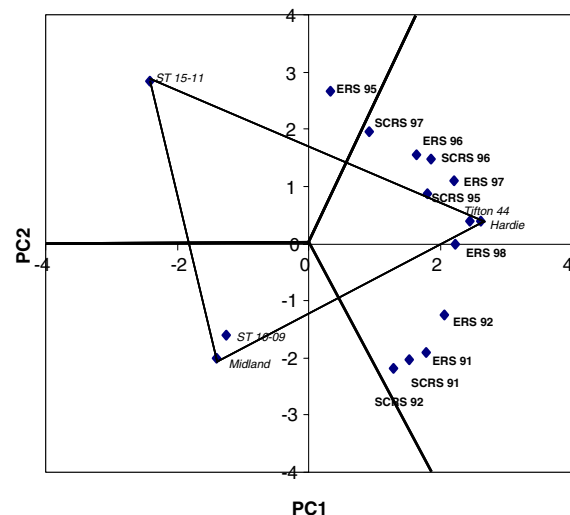

Fig. 1 GGE biplot of the bermudagrass trials conducted at Oklahoma State University from 1991 to 1998 at the Eastern Research Station and South Central Research Station (ERS and SCRS, respectively), constructed for examination of GE interaction
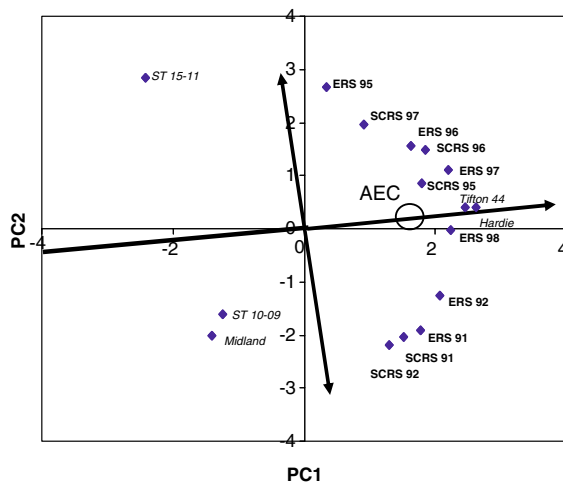
Fig. 2 GGE biplot of the bermudagrass trials conducted at Oklahoma State University from 1991 to 1998 at the Eastern Research Station and South Central Research Station (ERS and SCRS, respectively), constructed for examination genotype stability

the mean of the PC1 and PC2 scores for environments. An abscissa is formed by tracing a line through the AEC and the biplot origin; an AEC ordinate is drawn perpendicular to the AEC and through the biplot origin. Genotypes are ranked along the AEC abscissa, with the arrow pointing to greater mean yield (Fig. 2), while genotypes exhibiting the greatest projection onto the AEC are the least stable. The AEC ordinate (Fig. 2) arrows point in the directions of greater genotype instability. Hence, the most desirable genotypes are located near upper positive coordinates of and with small projection onto the AEC-abscissa.

Results and discussion

The ANOVA indicated that the effects of environment (year × location combination) (E), genotype (G), and GE interaction were highly significant ($P \leq 0.01$) on dm yield of the bermudagrass genotypes (Table 1). This indicated variations among the bermudagrass genotypes for dm yield and variations in environmental and GE effects. Environment effect accounted for most of the sums of squares indicating the substantial effect of environment on the dm yield performance of the five genotypes evaluated in this study. Significant GE effects demonstrated that the genotypes responded differently to variations in environmental conditions.

Table 1 Analyses of variance for bermudagrass dry matter yield in 11 environments (combinations of locations and years) in Oklahoma

Sources of variation	df	Mean squares
Environments (E)	10	441.79**
Replications within E	33	6.05**
Genotype (G)	4	83.46**
G × E	40	10.53**
Error	132	2.97

** Indicates significance at 0.01 level of probability

Table 2 Mean yield and five nonparametric statistics for dry matter yield of five bermudagrass cultivars in 11 environments (combinations of locations and years) in Oklahoma

Genotype	Yield (Mg ha^{-1})	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	Rank-sum
Hardie	21.24	0.87	0.62	3.57	4.20	3
Tifton 44	21.18	1.02	0.89	4.67	3.81	3
ST 10-09	19.31	1.38	1.62	4.34	2.78	6
Midland	18.84	0.95	0.69	1.69	1.78	8
ST 15-11	18.23	1.56	1.87	5.29	3.54	10

Table 3 Ranks of five bermudagrass cultivars based on dry matter yield, five nonparametric stability statistics, and biplot methodology

Genotype	Yield (Mg ha^{-1})	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	Rank-sum	Biplot
Hardie	1	1	1	2	5	1.5	1
Tifton 44	2	3	3	4	4	1.5	2
ST 10-09	3	4	4	3	2	3	3
Midland	4	2	2	1	1	4	4
ST 15-11	5	5	5	5	3	5	5

Avis et al. (1980) and Chakroun et al. (1990) reported similar results.

The numerical values of the test statistics for the different nonparametric methods are presented in Table 2. Based on these values the genotypes are ranked (Table 3), where the lowest rank for dm yield corresponds to the highest yielding genotype; regarding the stability statistics, the lowest rank means the most stable genotype across environments.

The nonparametric statistics $S_i^{(1)}$ and $S_i^{(2)}$ are in complete agreement in ranking the five genotypes where Hardie is identified as the most stable genotype

across environments. The rank-sum method also identified Hardie as the most stable genotype. Hardie also has the highest mean yield among the five genotypes. However, in ranking the rest of the genotypes, the rank-sum method was not in complete agreement with the $S_i^{(1)}$ and $S_i^{(2)}$ statistics. The rank-sum method also placed the genotype Tifton 44 in the same rank as Hardie for stability. It should be noted here that although Hardie and Tifton 44 ranked 1 and 2, respectively for dm yield, their mean yields are very similar (21.24 Mg ha^{-1} for Hardie and 21.18 Mg ha^{-1} for Tifton 44). Midland was ranked as the most stable genotype by both $S_i^{(3)}$ and $S_i^{(6)}$ statistics while $S_i^{(1)}$ and $S_i^{(2)}$ identified Midland as the second most stable genotype. The rankings of the remainder of the genotypes by $S_i^{(3)}$ and $S_i^{(6)}$ are also not in complete agreement with $S_i^{(1)}$ and $S_i^{(2)}$ or the rank-sum method. It is interesting to note that the rankings realized from Kang's rank-sum method are in complete agreement with the yield rank of the genotypes. Yue et al. (1997) concluded that Kang's rank-sum method reconciles the approaches of selecting exclusively for yield or stability.

The biplot explained 87.58% of the variation in the study (58.64 and 28.93% for PC1 and PC2, respectively). Crossover-type GE interaction was evident in the GGE biplot constructed for examination of GE interaction (Fig. 1). That is, genotypes are partitioned into three distinct sectors of the biplot. Tifton 44 and Hardie were the highest yielding genotypes (ranking either first or second) in all environments except ERS 95 (Fig. 1), in which ST-15 was the highest yielding genotype. Genotypes Midland and ST 10-09 were not the highest yielding genotypes in any of the 11 location–year environments. These findings pertaining to GE interaction are in agreement with those from the ANOVA. Examination of the biplot constructed for genotype stability analysis (Fig. 2) evinces that genotypes Hardie and Tifton 44 are located on the AEC abscissa at approximately 2.5 and at approximately 0.4 on the AEC ordinate. Hence, according to the biplot, these two genotypes exhibited the highest mean yield over environments and exhibited the greatest stability for dm yield. Mean yield and the estimates of the five nonparametric statistics are presented in Table 2; the ranking of all genotypes for each stability assessment method are listed in Table 3.

These results of the biplot analysis for genotype stability and Kang's rank-sum method are in complete agreement. Studies have indicated a high correlation of the ranking of genotypes for stability between the biplot and Kang's rank-sum method (Yan and Kang 2003). Moreover, both of the methods showed complete congruence between stability rankings and mean yield, indicating that these methods are better indicators of the dynamic concept of stability (Yan and Kang 2003).

From examination of the formulae for the $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$ nonparametric statistics it can readily be noted that the final ranking for any genotype or cultivar is influenced by the number of genotypes assayed. For the statistics that employ deviations of ranks from the mean deviations of ranks, there is a great potential for the numerator of the equations to enlarge disproportionately to the denominator as the number of genotypes increases and the number of environments remains static. The potential for disparity between the numerator and denominator of the nonparametric equations to develop increases as the number of genotypes exhibiting similar yields across all environments increases. Hence, final calculated values may be very sensitive to the number of genotypes in the sample, making these statistics much more reliable predictors of genotype stability as the number of genotypes in the sample increases.

The GGE biplot method of assaying genotype stability involves the elucidation of orthogonal PC scores (via singular value decomposition) for genotypes and environments from which GE can be recovered. This allows for the mean yield of genotypes to be plotted in reference to the AEC abscissa without contingent information from the PC scores for the main effect of genotypes. Hence, stability analysis via the visual biplot method is not sensitive to the number of genotypes assayed.

Conclusions

The results from this study suggest that a significant degree of GE interaction exists between the five genotypes assayed over the 11 year–location environments. The results further suggested that the dynamic concept of stability was best predicted by the biplot and by Kang's rank-sum nonparametric statistic. Examination of the formulae for the $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$ nonparametric statistics coupled with the results from these statistics for the five genotypes

assayed herein suggests that these statistics are potentially very sensitive when a small sample of genotypes is assayed. Based on this study, we conclude that for small numbers of perennial crop genotypes, especially bermudagrass, the best predictor of genotype stability would be the biplot method, with concomitant analysis via Kang's rank-sum method.

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